

IN THE CLAIMS

1. (previously amended) A method of making a plant artificial chromosome, comprising:

(a) preparing recombinant protoplasts of a first plant species containing an exogenous nucleic acid;

(b) irradiating the protoplasts of (a), thus producing chromosome fragments of chromosomes contained in the recombinant protoplasts;

(c) fusing the recombinant protoplasts of (b) with protoplasts of a second plant species to produce fused protoplasts, wherein the first and second plant species may be the same or different; and

(d) identifying fused protoplasts of (c) or cells derived from the fused protoplasts of (c) that contain chromosome fragments containing the exogenous nucleic acid, and that exhibit normal plant chromosomal activities.

2. (previously amended) The method of claim 1 wherein (b) comprises irradiating the protoplasts with gamma radiation.

3. (cancelled)

4. (currently amended) The method of claim 3—1 wherein said irradiating is at the chemical agent is selected from the group consisting of calicheamicin, esperamicin, dynemicin or and neocarzinostatin.

5. (original) The method of claim 1 wherein said identifying of (d) comprises pulsed field gel electrophoresis.

6. (original) The method of claim 1 wherein said second plant species is the same as said first plant species.

7. (original) The method of claim 1 wherein said second plant species is a member of the same family as said first plant species.

8. (original) The method of claim 1 wherein said first plant species is a monocot.

9. (original) The method of claim 1 wherein said first plant species is a dicot.

10. (cancelled)

11. (original) The method of claim 1 further comprising (f) regenerating a whole plant from the fused protoplasts or plant cells identified in claim 1(d).

12. (previously presented) The method of claim 1 wherein the exogenous nucleic acid comprises at least one recombination site recognized by a site specific recombinase.

13. (original) The method of claim 1 wherein the exogenous nucleic acid comprises at least one restriction site.

14. (original) The method of claim 1 wherein the exogenous nucleic acid comprises at least one coding region.

15. (previously amended) The method of claim 1 wherein the exogenous nucleic acid comprises at least one sequence comprising a yeast chromosomal element.

16. (previously amended) The method of claim 1 wherein the exogenous nucleic acid comprises a yeast artificial chromosome.

17. (cancelled)

18. (previously amended) A method of preparing a transgenic plant comprising:

(a) preparing recombinant protoplasts of a first plant species containing an exogenous nucleic acid;

(b) irradiating the protoplasts of (a), thus producing chromosome fragments of chromosomes contained in the recombinant protoplasts;

(c) fusing the recombinant protoplasts of (b) with protoplasts of a second plant species to produce fused protoplasts, wherein the first and second plant species may be the same or different;

(d) identifying fused protoplasts of (c) or cells derived from the fused protoplasts of (c) that contain

chromosome fragments that exhibit normal plant chromosomal activities; and

(e) regenerating a whole plant from the protoplasts or cells identified in (d) that contain said chromosome fragments containing the exogenous nucleic acid, and that exhibit normal plant chromosomal activities.

19. (currently amended) A method of making a plant artificial chromosome, comprising:

(a) producing transformed plants of a first plant species containing an exogenous nucleic acid;

(b) ~~irradiating the protoplasts of the transformed plants~~ of (a), thus producing chromosome fragments of chromosomes of said first plant species;

(c) crossing said first plant species containing the chromosome fragments with a second plant species to produce hybrid plant species wherein said first and second plant species may be the same or different; and

(d) identifying hybrid plant species of (c) or cells or protoplasts thereof containing at least one chromosome fragment containing the exogenous nucleic acid, and that exhibits normal plant chromosomal activities.

20. - 35. (cancelled)

36. (previously presented) The method of claim 15, wherein the yeast chromosomal element comprises a first centromeric sequence functional in a yeast cell.

37. (previously presented) The method of claim 36, wherein the chromosome fragments further comprise a second centromeric sequence functional in a plant cell.

38. (previously presented) A method of making a plant artificial chromosome, comprising:

(a) preparing recombinant protoplasts of a first plant species containing an exogenous nucleic acid comprising a selectable marker gene;

(b) producing chromosome fragments of chromosomes contained in the recombinant protoplasts;

(c) fusing the recombinant protoplasts of (b) with protoplasts of a second plant species to produce fused protoplasts, wherein the first and second plant species may be the same or different; and

(d) identifying fused protoplasts of (c) or plant cells derived therefrom that contain chromosome fragments that contain the exogenous nucleic acid and that exhibit normal plant chromosomal activities.

39. (previously presented) The method of claim 38, wherein the exogenous nucleic acid further comprises at least one yeast chromosomal element.

40. (previously presented) The method of claim 39, wherein the yeast chromosomal element comprises a first centromeric sequence functional in a yeast cell.

41. (previously presented) The method of claim 40, wherein the chromosome fragments comprise a second centromeric sequence functional in a plant cell.

42. (cancelled)

43. (currently amended) A method of making a plant artificial chromosome, comprising:

(a) preparing recombinant protoplasts of a first plant species containing an exogenous nucleic acid;

(b) regenerating a whole plant from the recombinant protoplasts;

(c) selecting cells obtained from the regenerated plant of (b) that contain the exogenous nucleic acid;

(d) irradiating the cells of (c), thus producing chromosome fragments of chromosomes contained in the cells of (c);

(e) fusing protoplasts of containing the chromosome fragments from the cells of (d) with protoplasts of a second

plant species to produce fused protoplasts, wherein the first and second plant species may be the same or different; and

(f) identifying fused protoplasts of (e) or cells derived from the fused protoplasts of (e) that contain chromosome fragments containing the exogenous nucleic acid, and that exhibit normal plant chromosomal activities.

44. (previously presented) The method of claim 43, wherein said nucleic acid comprise a marker gene.